Antiviral Research, 20 (1993) 317–331

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AVR 00618

In vitro antiviral activity of polyoxomolybdates. Mechanism of inhibitory effect of PM-104 $(NH_4)_{12}H_2(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4) \cdot 13H_2O$ on human immunodeficiency virus type 1

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(Received 5 May 1992; accepted 12 November 1992)

Summary

A screening for inhibitors of human immunodeficiency virus type 1 (HIV-1) among various types of isopolyoxomolybdates and heteropolyoxomolybdates was carried out by using an in vitro assay system measuring the cytopathogenicity of HIV-1 in $CD4^+$ human MT-4 cells. A novel heteropolyoxomolybdate named PM-104 with the chemical formula $(NH_4)_{12}H_2(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4) \cdot 13H_2O$ was found to be associated with potent anti-HIV-1 activity.

PM-104 interferes with virus infection at a very early step such as adsorption and/or penetration into the cells. In addition to the cytopathic effect of HIV-1 on MT-4 cells, syncytium formation between mock-infected MOLT-4 cells and MOLT-4 cells chronically infected with either HIV-1 or HIV-2 is suppressed by PM-104. PM-104 also blocks the replication of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). The antiviral properties of PM-104 could be attributed to the combined effect of europium atoms and its peculiar three-dimensional anion structure.

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Polyoxomolybdate; Polyoxometalate; Human immunodeficiency virus type 1; Herpes simplex virus

Introduction

The principal units that make up most polyoxometalates, condensed oligomeric aggregate anions of transition-metal oxide, are MO_6 octahedra, in which M is usually tungsten or molybdenum, linked together by a single oxygen atom (corner-sharing) or two oxygen atoms (edge-sharing). Isopolyoxometalates are constituted of metal and oxygen atoms whilst heteropolyoxometalates contain one or more 'heteroatoms (X)' in addition to parental metal and oxygen atoms. The polyoxometalates consisting of molybdenum and tungsten atoms are generally referred to as polyoxomolybdates and polyoxotungstates, respectively.

HPA 23 [$(NH_4)_{17}Na$ ($NaSb_9W_{21}O_{86}$)] was the first heteropolyoxotungstate (HPOT) applied to clinical trials for patients with acquired immune deficiency syndrome (AIDS) which is caused by a retrovirus, human immunodeficiency virus type 1 (HIV-1) (Moskovitz et al., 1988; Rozenbaum, 1985; Vittecoq et al., 1988), since it had been well known as an antiretroviral agent in animal models and as an inhibitor of reverse transcriptases. The latter plays a pivotal role in the life cycle of retroviruses (Dormont, 1988; Jasmin et al., 1974, 1975).

Recently, we observed marked inhibition of the replication of HIV-1 by some Keggin polyoxotungstates represented by PM-19 $K_7(PTi_2W_{10}O_{40}) \cdot 6H_2O$ and related compounds such as PM-48 $K_{13}(Eu(SiW_{11}O_{39})_2) \cdot 30H_2O$ which has a deficient dimerized Keggin structure (Inouye et al., 1990; Take et al., 1991). In addition to HIV-1, herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) were found to be susceptible to the suppressive effect of Keggin HPOTs (Fukuma et al., 1991).

In contrast, Hill et al. (1990) and Take et al. (1991) reported the lack of potent antiviral activity of structurally characterized polyoxomolybdates. Recently, we described the anti-HIV-1 activity of PM-104 $(NH_4)_2H_2(Eu_4(MoO_4)\ (H_2O)_{16}(Mo_7O_{24})_4) \cdot 13H_2O$ (Inouye et al., 1991).

In this paper, we describe the results of the anti-HIV-1 screening of various types of polyoxomolybdates as well as the mode of action by which PM-104 interferes with the replication of HIV-1.

Materials and Methods

Chemicals

The following compounds were prepared and purified as described previously: $K_5(BW_{12}O_{40}) \cdot 15H_2O$ (PM-1) (Rocchicciali-Deltcheff et al., 1983; Yamase and Watanabe, 1986); $Na_6(NiMo_9O_{32}) \cdot nH_2O$ (PM-5) and

$Na_6(MnMo_9O_{32}) \cdot nH_2O$ (PM-6) (Baker and Weakley, 1966); $(iPrNH_3)_6(Mo_7O_{24}) \cdot 3H_2O$ (PM-8) (Yamase and Ikawa, 1977; Yamase et al., 1981; Ohashi et al., 1982); $Na_6(Mo_6V_2O_{26}) \cdot nH_2O$ (PM-15) (Björnberg, 1979); $K_7(PTi_{12}W_{10}O_{40}) \cdot 6H_2O$ (PM-19) (Domaille and Knoth, 1983; Ozeki and Yamase, 1991); $Na_5(IMo_6O_{24}) \cdot 3H_2O$ (PM-32) (Filowitz et al., 1979); $(NH_4)_6(Cr_2Mo_{12}O_{42}) \cdot 20H_2O$ (PM-33) (Rosenheim and Schwer, 1914); $(NH_4)_3H_6(CoMo_6O_{24}) \cdot 7H_2O$ (PM-41) (Shibata, 1966); $Na_3H_6(CrMo_6O_{24}) \cdot 8H_2O$ (PM-42) (Perloff, 1970); $K_{13}(Eu(SiW_{11}O_{39})_2) \cdot 30H_2O$ (PM-48) (Ballardini et al., 1984); $K_7(PMo_2W_9O_{39}) \cdot 19H_2O$ (PM-62), $Na_3H_6(PMo_9O_{34}) \cdot 13H_2O$ (PM-64), $K_3(PMo_3W_9O_{40}) \cdot 25H_2O$ (PM-66) and $K_3(PMo_9W_3O_{40}) \cdot 5H_2O$ (PM-67) (Massart et al., 1977); $K_{15}H_3(Eu_3(H_2O)_3(W_5O_{18})_3(SbW_9O_{33})) \cdot 25 \cdot 5H_2O$ (PM-69) (Yamase et al., 1990); $(NH_4)_{12}H_2(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4) \cdot 13H_2O$ (PM-104) (Naruke et al., 1991); and $(Me_4N)_2(NH_4)_8(Mo_{14}O_{46}) \cdot 8H_2O$ (PM-112) (Yamase, 1991). The preparative procedures of $(iPrNH_3)_5H(Mo_7O_{22}(O_2)_2) \cdot 3H_2O$ (PM-26), $(iPrNH_3)_4(Se_2Mo_5O_{21}) \cdot 3H_2O$ (PM-117) and $(iPrNH_3)_6(P_2Mo_5O_{23}) \cdot 5H_2O$ (PM-118) are similar to those of $K_6Mo_7O_{22}(O_2)_2 \cdot 8H_2O$ (Larking and Stomberg, 1972), $(NH_4)_4(Se_2Mo_5O_{21}) \cdot 3H_2O$ (Ichita et al., 1986) and $Na_6(P_2Mo_5O_{23}) \cdot 13H_2O$ (Strandberg, 1973), respectively. IR spectra of PM-1, -15, -19, -32, -33, -41, -42, -48, -62, -64, -66, -67, -107 and -118; [^{31}P] and [^{183}W] NMR spectra of PM-19, -66, -67 and -118; and electronic spectra of PM-5 and -6 let us confirm the anions identical with those given by each reference. Single-crystal X-ray diffraction analyses were used for the structural characterization of PM-8 (Ohashi et al., 1982), -26 (Yamase, unpublished result), -69 (Yamase et al., 1990), -104 (Naruke et al., 1991) and -112 (Yamase et al., 1991). $(NH_4)_{12}H_2(Ln_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4) \cdot 13H_2O$ (Ln = Nd, Pr, La, Ce and Sm; PM-113, -114, -115, -116 and -119, respectively) were prepared by a slight modification of the procedure for PM-104 and characterized by their IR spectral patterns at the Mo-O stretching regions, which is similar to that of the crystallographically characterized PM-104 (Naruke et al., 1991). In addition, the coordination of Sm^{3+} for PM-119 was confirmed by its photoluminescence spectrum characteristic of Sm^{3+}. $K_5NaH(Mo_9V_3O_{38}) \cdot 7H_2O$ (PM-13) and $(NH_4)_{17}Na(NaSb_9W_{21}O_{86}) \cdot 14H_2O$ (HPA 23) were generously provided by Prof. L. Pettersson (University of Umeå, Sweden) and Prof. A. Tézé (Université Pierre et Marie Curie, France), respectively.

Analytical grade $Na_2MoO_4 \cdot 2H_2O$, $(NH_4)_6(Mo_7O_{24}) \cdot 4H_2O$ and $Na_3(PMo_{12}O_{40}) \cdot nH_2O$ were commercially obtained and used without further purification. The anions for PM compounds listed in Table 1 except for PM-5, -6 and -66 are stable at high concentrations and assumed to be stable in diluted solutions ($\sim \mu M$) at physiological pH. PM-5, -6 and -66 are unstable in aqueous solutions (Baker and Weaker, 1966; Massart et al., 1977).

Acyclovir (ACV) was obtained from The Wellcome Foundation. Dextran sulfate (DS, average mol.wt. ca. 8000) was purchased from Sigma Chemical. 3′-Azido-2′,3′-dideoxythymidine (AZT) was a product of Burroughs Wellcome. All other materials used in this paper are commercial products of analytical grade.

TABLE 1

Toxicity and anti-HIV-1 activity of polyoxomolybdates

Compound	Chemical formula	CC_{50} (μg/ml)	EC_{50} (μg/ml)	TI_{50}[a]
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	>800	NA[b]	
Paramolybdate derivatives				
Ammonium heptamolybdate	$(NH_4)_6[Mo_7O_{24}] \cdot 4H_2O$	570	NA	
PM-8	$(iPrNH_3)_6[Mo_7O_{24}] \cdot 3H_2O$	260	NA	
PM-26	$(iPrNH_3)_5H[Mo_7O_{26}] \cdot 3H_2O$	280	NA	
PM-112	$(Me_4N)_2(NH_4)_8[Mo_{14}O_{46}] \cdot 8H_2O$	500	NA	
Keggin structure; 1:12[c], tetrahedral[d]				
PM-102	$Na_3[PMo_{12}O_{40}] \cdot nH_2O$	750	NA	
PM-66	$K_3[PMo_3W_9O_{40}] \cdot 25H_2O$[e]	150	83	1.8
PM-67	$K_3[PMo_9W_3O_{40}] \cdot 5H_2O$[e]	240	NA	
Lacunary Keggin structure; 1:11[c], tetrahedral[d]				
PM-62	$K_7[PMo_2W_9O_{39}] \cdot 19H_2O$[d]	160	82	2.0
Trivacant Keggin structure; 1:9[c], tetrahedral[d]				
PM-64	$Na_3H_6[PMo_9O_{34}] \cdot 13H_2O$	430	NA	
Anderson structure; 1:6[c], octahedral[d]				
PM-32	$Na_5[IMo_6O_{24}] \cdot 3H_2O$	68	NA	
PM-41	$(NH_4)_3H_6[CoMo_6O_{24}] \cdot 7H_2O$	49	NA	
PM-42	$Na_3H_6[CrMo_6O_{24}] \cdot 8H_2O$	430	NA	
Polyoxomolybdolanthanoates, tricapped trigonal prismatic[d]				
PM-104	$(NH_4)_{12}H_2[Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot 13H_2O$	300	4.4	68
PM-113	$(NH_4)_{12}H_2[Nd_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot nH_2O$	430	NA	
PM-114	$(NH_4)_{12}H_2[Pr_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot nH_2O$	320	NA	
PM-115	$(NH_4)_{12}H_2[La_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot nH_2O$	500	NA	
PM-116	$(NH_4)_{12}H_2[Ce_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot nH_2O$	440	NA	
PM-119	$(NH_4)_{12}H_2[Sm_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4] \cdot nH_2O$	210	NA	
Miscellaneous tetrahedral[d]				
PM-15	$Na_6[Mo_6V_2O_{26}] \cdot nH_2O$	4.2	NA	
PM-117	$(iPrNH_3)_4[Se_2Mo_5O_{21}] \cdot nH_2O$	4.4	NA	
PM-118	$(iPrNH_3)_4[P_2Mo_5O_{23}] \cdot nH_2O$	460	NA	
Miscellaneous, octahedral[d]				
PM-5	$Na_6[NiMo_9O_{32}] \cdot nH_2O$[e]	14	NA	
PM-6	$Na_6[MnMo_9O_{32}] \cdot nH_2O$[e]	33	NA	
PM-13	$K_5NaH[Mo_9V_3O_{38}] \cdot 7H_2O$	4.4	NA	
PM-33	$(NH_4)_6[Cr_2Mo_{12}O_{42}] \cdot 20H_2O$	300	NA	
Heteropolyoxotungstates				
Keggin structure				
PM-1	$K_5[BW_{12}O_{40}] \cdot 15H_2O$	190	18	11
PM-19	$K_7[PTi_2W_{10}O_{40}] \cdot 6H_2O$	270	4.0	68

Lacunary Keggin structure				
PM-48	$K_{13}[Eu(SiW_{11}O_{39})_2] \cdot 30H_2O$	54	3.4	16
Mixed ligand polyoxotungstoeuropate				
PM-69	$K_{15}H_3[Eu_3(H_2O)_3(W_5O_{18})_3(SbW_9O_{33})] \cdot 25 \cdot 5H_2O$	150	NA	
Miscellaneous				
HPA 23	$(NH_4)_{17}Na[NaSb_9W_{21}O_{86}] \cdot 14H_2O$	7.5	NA	
Others				
AZT		4.2	0.0042	1000
DS		>800	2.2	>360

[a]$TI_{50} = CC_{50}/EC_{50}$.
[b]EC_{50} was not available since the maximum protection of MT-4 from the cytopathic effect of HIV-1 is 800 μg/ml less than 50%. The highest concentration used was ...
[c]Stoichiometry of the heteroatom (central atom): peripheral atom.
[d]Shape of coordination around a central atom.
[e]Unstable at physiological pH in aqueous solution (Massart et al., 1977).

Cells

MT-4 cells (Miyoshi et al., 1982), which are highly sensitive to the cytopathic effect of HIV-1, were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS); penicillin, 100 IU/ml; and streptomycin, 100 μg/ml. Uninfected MOLT-4 cells, HIV-1 infected MOLT-4 cells (MOLT-4/HTLV-III_b, MOLT-4/ARV) (Koyanagi et al., 1985, 1986; Popovic et al., 1984; Levy et al., 1984) and HIV-2(GH-1)-infected MOLT-4 cells (MOLT-4/0650) (Ishikawa et al., 1988) were cultured in the same medium. Vero cells were obtained from Flow Lab., USA and cultured in Eagle's minimum essential medium (MEM) supplemented with 5% FCS.

Viruses

A virus stock of HTLV-III_b was obtained from the culture supernatant of MOLT-4/HTLV-III_b, titrated in MT-4 cells (10^4 $TCID_{50}$/ml) and stored at −80°C until use. Other viruses used in this study were as follows: herpes simplex virus type 1 (HSV-1), KOS and Hayashida strains; herpes simplex virus type 2 (HSV-2), 169, Tomioka and ACV-resistant YS-4C-1 strains; varicella-zoster virus (VZV); and polioencephalomyelitis virus type 1, Mahoney strain.

Inhibition of virus infection

Assay for the anti-HIV-1 activity was based on the inhibition of virus-induced cytopathogenicity (cytopathic effect, CPE) in MT-4 cells. MT-4 cells were suspended in the culture medium at 1×10^5 cells/ml and infected with HIV-1 at a multiplicity of infection of 0.01 for 1 h at 37°C. Cells were washed and resuspended in the same medium at 5×10^4 cells/ml and portions (100 μl) of cell suspension were mixed with 100 μl of various concentrations of the test compounds in the wells of flat-bottomed 96-well microtiter trays.

After 6 days of incubation at 37°C, the cell viability was determined by 3-

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay (Pauwels et al., 1988). The viability of mock-infected host cells was determined in order to evaluate the cytotoxicity of the test compounds.

For the other viruses, the antiviral activity of the test compounds evaluated by a plaque-reduction assay. The details were reported previously by Fukuma et al. (1991). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration required for 50% inhibition of the growth of Vero cells under the assay conditions described previously (Fukuma et al., 1991).

MTT assay

Twenty-five μl of 10 mg/ml MTT in phosphate-buffered saline was added to each well of the microtiter trays, and formazan production was allowed to proceed for 4 h. Removal of 200 μl of the medium was followed by the addition of 100 μl of acidified isopropanol (0.04 N HCl) to solubilize the formazan dye. Quantitative analysis of the cell viability was accomplished by measurement of the optical density at 595 nm (measurement wave-length) and 655 nm (reference wavelength). The calculation of HIV-1-induced cytopathic effect (CPE) was based on the viability of mock-infected and HIV-1-infected cultures (Harada et al., 1985). The percent protection was calculated according to the method of Pauwels et al. (1988). The 50% effective concentration (EC_{50}) was defined as the concentration at which 50% protection was obtained. Definition of CC_{50} was the concentration of a compound that reduced the cell viability of the mock-infected MT-4 cells by 50%.

Inhibition of syncytium formation

Syncytium formation was assayed by the method of Tochikura et al. (1988) with some modifications. Briefly, MOLT-4 cells and persistently HIV-infected MOLT-4 cells (MOLT-4/HTLV-III_b, MOLT-4/ARV or MOLT-4/0650) were cultured at the final cell density of 2.5×10^5 cells/ml in a mixture of 2:1 or individually in the presence of various concentrations of test compounds at 37°C for 20 h. Control wells received either MOLT-4 cells, chronically HIV-infected MOLT-4 cells or the 2:1 mixture in the absence of test compounds at the same cell density as those added with test compounds. The numbers of cells were determined in Coulter counter Model DN (Coulter Electronics). The fusion index (FI) was defined as follows:

FI = ((Cell number in MOLT-4 well $\times$ 2 + Cell number in MOLT-4/HTLV-III_b well)/(Cell number in mixed-culture well $\times$ 3)) $-$ 1.0.

The control FI ranged 0.7 to 0.9. The % reduction in $FI = (1 - FI_T/FI_C) \times 100$, where FI_T is the FI of the test compound and FI_C is that of the control.

Results

Inhibition of HIV-1-induced cytopathogenicity by polyoxomolybdates

Five isopolyoxomolybdates (IPOMs) were examined for their anti-HIV-1

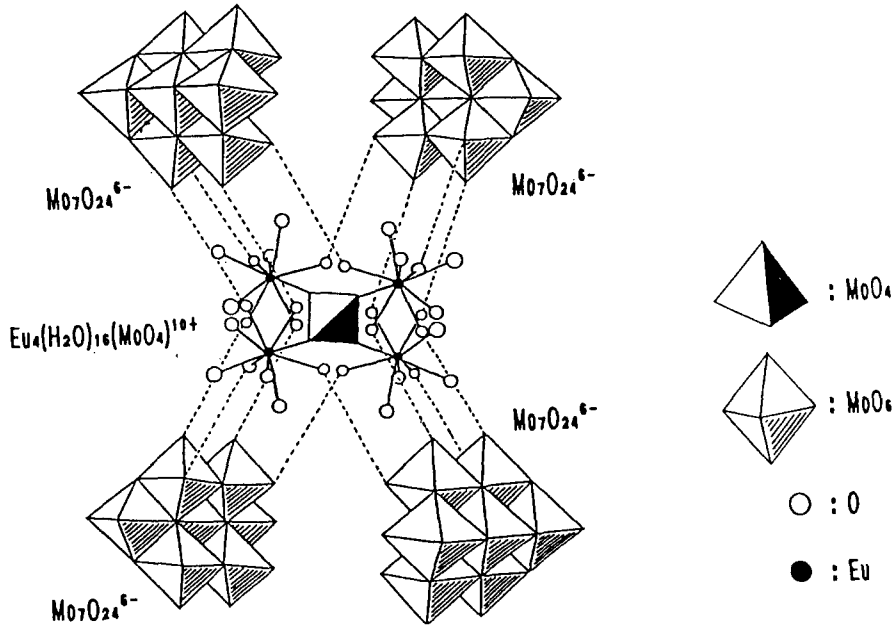

Fig. 1. Structural features of the anion in PM-104, $(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4)^{14-}$

activities. None of them protected MT-4 cells from HIV-1 CPE (Table 1).

Like IPOMs, heteropolyoxomolybdates (HPOMs) (except for PM-104) are inactive against cytopathogenicity of HIV-1 (Table 1). PM-104 showed no toxicity up to a concentration of 200 μg/ml and at the concentrations of 12.5 μg/ml or higher PM-104 suppressed CPE induced by HIV-1 (Inouye et al., 1991). The TI_{50} values, i.e., the ratios of CC_{50}/EC_{50}, of PM-104 and AZT were 68 and 1000, respectively. Thus, the anti-HIV-1 activity of PM-104 was by far inferior to that of AZT [as could be expected from their mode of action (Inouye et al., 1991)] but it favorably compared with that of PM-19.

The three-dimensional structure of PM-104 anion is shown in Fig. 1. PM-104 consists of four europium atoms, one tetrahedral MoO_4 unit and four Mo_7O_{24} units. The Mo_7O_{24} unit is made up from seven MoO_6 octahedra in a structure identical to the anionic structures of ammonium heptamolybdate $(NH_4)_6(Mo_7O_{24})\cdot 4H_2O$ and PM-8 $(iPrNH_3)_6\ (Mo_7O_{24})\cdot 3H_2O$.

As shown in Table 1, both $(MoO_4)^{2-}$ and $(Mo_7O_{24})^{6-}$ failed to exhibit anti-HIV-1 activity. To study the contribution of europium atoms to the anti-HIV-1 activity of PM-104, five novel HPOMs were synthesized by replacing europium atoms in PM-104 with other lanthanide atoms: PM-113, neodymium (Nd); PM-114, praseodymium (Pr); PM-115, lanthanum (La); PM-116, cerium (Ce) and PM-119, samarium (Sm). Unlike PM-104, these compounds were devoid of anti-HIV-1 activity. Europium chloride showed inhibition of HIV-1 CPE in MT-4 cells at concentrations of 200 to 400 μg/ml (Fig. 2). There were no other metal salts (for example, $ZnCl_2$, $CaCl_2$, $MnCl_2$, $MgCl_2$, $CoCl_2$, $NdNO_3$,

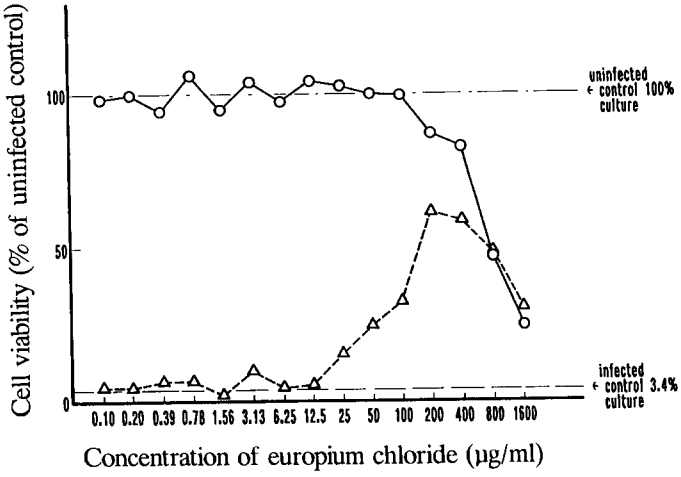

Fig. 2. Effect of europium chloride on the cytopathogenicity of HIV-1. MT-4 cells were infected with HIV-1 (moi = 0.01) for 1 h. $EuCl_3$ was added to the culture and the cell number was adjusted to 5×10^3 cells/ml in a total volume of 200 μl. The cell viability was measured at 6 days after the virus infection. ○—○, Uninfected and drug-treated culture; △- - -△, HIV-1-infected and drug-treated culture.

$LaNO_3$) that showed anti-HIV-1 activity. The 50% lethal dose (LD_{50}) of PM-104 for mice was as follows: ca. 300 mg/kg (i.v.), ca. 500 mg/kg (i.p.) and >2000 mg/kg (p.o.).

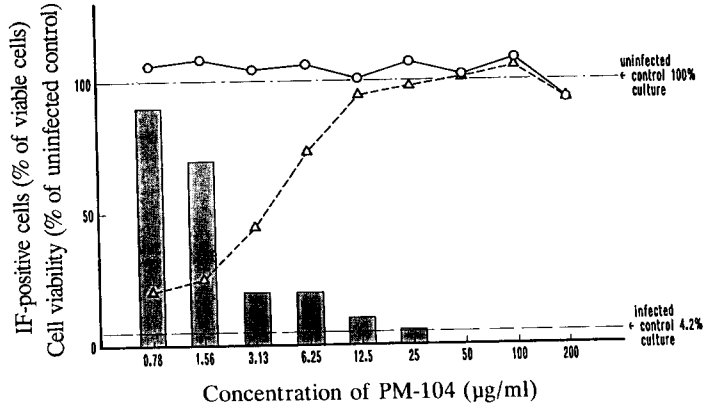

Fig. 3. Concentration dependence of the suppression of virus adsorption. The mixture of 500 μl of MT-4 cell suspension (2×10^5 cells/ml), 600 μl of varied concentrations of PM-104 (0, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, 200 and 400 μg/ml) and 100 μl of HIV-1 (1×10^4 $TCID_{50}$/ml) was incubated at 37°C for 1 h. The cells were washed and the cell number was adjusted to 5×10^3 cells/well in a total volume of 200 μl. On day 6, the cell viability (○—○, uninfected; △- - -△, HIV-1-infected) and % of IF-positive cells among HIV-1-infected population (■) were measured.

Mechanism of inhibition of HIV-1 replication by PM-104

The HIV-1-induced CPE was inhibited by PM-104 in a concentration-dependent fashion, when the host cells were treated with the compound during the 1 h HIV-1 adsorption period (Fig. 3). The higher the concentration of PM-104, the more significant the suppression of HIV-1-induced CPE. Complete inhibition of CPE was observed at concentrations higher than 12.5 μg/ml, while IF-positive cells were not detected at concentrations higher than 25 μg/ml. In contrast, there was no significant effect on CPE when MT-4 cells were treated with PM-104 at 100 μg/ml for 1 h before or after HIV-1 infection (Fig. 4). Thus, the presence of PM-104 during the virus adsorption was required for the effective inhibition of CPE. In an attempt to clarify the mechanism of action of PM-104, MT-4 cells were treated with a fixed amount of PM-104 for varying periods. Fig. 4 shows the time-dependence of reduction of CPE. HIV-1 proliferation was completely suppressed when PM-104 (100 μg/ml) was added to the cell culture simultaneously with HIV-1 or 1 min later. If HIV-1 infection in the absence of PM-104 lasted for 5 min or more, virus antigen-positive (Fig. 4) cells were observed. The percentage of virus antigen-positive cells passed the 50% point when treatment was delayed till 30 min after infection. On the basis of these findings, virus adsorption (and/or penetration) is considered to be the primary site of action of PM-104.

Inhibition of syncytium formation by PM-104

Cell-to-cell infection of HIV to MOLT-4 cells results in the formation of multinucleated giant cells (Tochikura et al., 1988). The effect of PM-104 on the syncytium forming ability of HIV was examined by using an in vitro assay

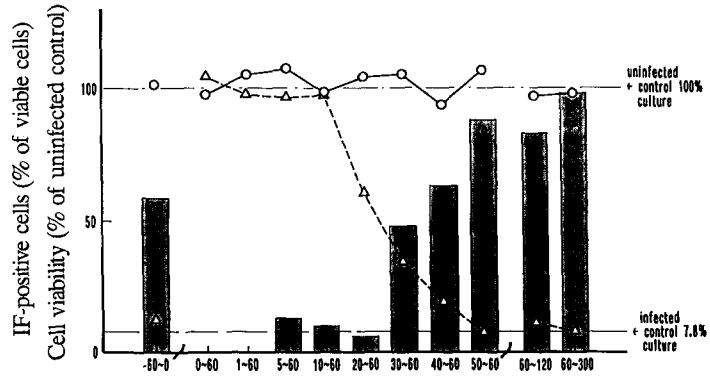

Fig. 4. Time dependence of the suppression of virus adsorption. The mixture of 500 μl of MT-4 cell suspension (2×10^5 cells/ml) and 100 μl of HIV-1 (1×10^4 $TCID_{50}$/ml) was incubated at 37°C for 1 h. The cells were treated with 100 μg/ml PM-104 for varied periods before, during or after the viral infection. The cells were washed and the cell number was adjusted to 5×10^3 cells/well in a total volume of 200 μl. On day 6, the cell viability (○—○, uninfected; △- - -△, HIV-1-infected) and % of IF-positive cells (■) were measured.

TABLE 2

Inhibition of syncytium formation by PM-104, DS and AZT

Compound	EC_{50} (μg/ml)		
	MOLT-4 + MOLT-4/III b	MOLT-4 + MOLT-4/ARV	MOLT-4 + MOLT-4/0650
PM-104	15	0.05	2.5
DS	5	0.9	0.01
AZT	>50	>50	>50

HIV-infected and uninfected MOLT-4 cells were cultured individually or in combination at the ratio of 1:2 for 20 h in the presence of PM-104, DS or AZT. Each culture was established with 2.5×10^5 cells in a total volume of 1 ml.

system consisting of uninfected MOLT-4 cells co-cultured with chronically HIV-infected MOLT-4 cells (MOLT-4/HTLV-III_B, MOLT-4/ARV or MOLT-4/0650). Syncytium formation in all three cases was significantly inhibited by PM-104 and DS, whereas no inhibition was shown by AZT at concentrations up to 50 μg/ml (Table 2).

Activities of polyoxomolybdates against herpes simplex viruses

The antiherpetic activities of PM-104 and PM-19 are shown in Table 3. As in the case of HIV-1, replication of these viruses was not inhibited by the HPOMs except for PM-104 (data not shown). There was no inhibition of VZV or poliovirus by any compound belonging to the group of the polyoxymetalates (HPOTs and HPOMs). This is in accord with previous findings (Fukuma et al., 1991).

Discussion

Antiviral activity of polyoxometalates seems to be determined by their anionic structures and the nature of the peripheral metals and central heteroatoms, individually or in combination. In contrast to the HPOTs, most HPOMs did not inhibit the replication of HIV-1 (Table 1) or HSV-1 and -2 (Table 3). The ratios of W to Mo atoms in the Keggin-structural molybdic and tungstic polyoxometalates determine the anti-HIV-1 activities: PM-102 (W:Mo

TABLE 3

Inhibitory effect of PM-19 and PM-104 on plaque formation of HSV-1 and HSV-2

Compound	CC_{50} (μg/ml)	EC_{50} (μg/ml)				
		HSV-1		HSV-2		
		KOS	Hayashida	169	Tomioka	YS-4C-1
Acyclovir (ACV)	480	0.07	0.06	0.06	0.06	6.4
PM-19	510	6.2	6.2	4.2	4.1	4.2
PM-104	310	6.0	5.5	3.3	3.6	4.3

ratio = 0:12) and PM-67 (3:9) are inactive, PM-66 (9:3) and PM-62 (9:2) is marginally active (TI_{50} about 2) and PM-1 (12:0) showed the highest activity (TI_{50} 11). Polarograms of W/Mo mixed Keggin-structural compounds at physiological pH diminish in height due to decomposition to mononuclear species, as was noted previously for PM-66 (Massart et al., 1977). As shown in Table 1, none of $(XMo_{12}O_{40})^{n-}$, $(Mo_7O_{24})^{6-}$ and MoO_4^{2-} is active against HIV-1. Furthermore, $(XW_{12}O_{40})^{n-}$ is active against HIV-1, while the trivacant form of $(PW_9O_{34})^{9-}$ is inactive. In conjunction with the fact that $(PTi_2W_{10}O_{40})^{7-}$ for PM-19 is stable at physiological pH and potentially active against HIV-1, therefore, treatment of the hydrolytically sensitive W/Mo mixed Keggin-structural compounds at experimental concentrations may to a very small part lead to the $(PW_{12}O_{40})^{3-}$ form, as suggested by the activities of PM-102 (W/Mo = 0/12), −67 (3/9), −66 (9/3) and −62 (9/2) (in order of increasing activity).

HPOM PM-104 has a potent anti-HIV-1 activity comparable to that of HPOT PM-19. PM-104 $(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4)^{14-}$ (Fig. 1) consists of four europium atoms, one tetrahedral MoO_4, four Mo_7O_{24} units and sixteen H_2O molecules (Naruke et al., 1991). Neither MoO_4^{2-} nor $Mo_7O_{24}^{6-}$ are inhibitory to HIV-1 (Table 1); however, europium chloride shows a slight suppressive effect on HIV-1 (Fig. 2), although its TI_{50} and maximum % of inhibition are as low as 4 and 65%, respectively. The PM-104-like compounds in which europium was replaced with the other lanthanide metals, i.e., PM-113 (Nd), −114 (Pr), −115 (La), −116 (Ce) and −119 (Sm), were hardly inhibitory to HIV-1. HPOTs, PM-48 K_{13} $(Eu(SiW_{11}\text{-}O_{39})_2 \cdot 30H_2O$ and PM-69 $K_{15}(H_3Eu_3(H_2O)_3(W_5O_{18})_3(SbW_9O_{33})) \cdot 25 \cdot 5H_2O$, also contain europium atoms (Table 1). The antiviral activity of PM-48, in which europium atom is coordinated by two 'lacunary Keggin' polyoxotungstates, might be attributed to its anionic structure rather than the europium atom, because PM-69 containing three europium atoms in the anion had no effect on the growth of HIV-1. From these observations, the potent anti-HIV-1 activity of PM-104 may be attributed to an amplification of the activity of Eu^{3+} by the coordination of specific polyoxomolybdate ligands.

As in the case of PM-19 (Take et al., 1991), PM-104 inhibits HIV-1 infection by interfering with the very early steps such as virus adsorption (and/or penetration) into the target cells (Fig. 3). Both compounds also show activity against HSV (Table 3), but not VZV or poliovirus type 1 (data not shown).

Sulfated polysaccharides such as dextran sulfate and heparin generated much interest when their inhibitory effect on the replication of HIV-1, attributed to an inhibition of HIV-1 adsorption to the cell membrane, was recognized (Ito et al., 1987; Ueno and Kuno, 1987; Baba et al., 1988a,b,c; Mitsuya et al., 1988). These compounds have been known as inhibitors of HSV and other viruses for more than two decades (De Somer et al., 1968). Recently, Baba et al. (1988a) re-evaluated their antiviral activities and found that sulfated polysaccharides are potent inhibitors of various types of enveloped viruses. In contrast, VZV is not sensitive to polyoxometalates (Fukuma et al., 1991), probably because

antiviral assays with VZV are carried out with cell-associated (rather than cell-free) virus.

The coculture of HIV-1-infected cells and the uninfected $CD4^+$ cells result in syncytium formation leading to a selective destruction of uninfected cells (Baba et al., 1990). Approximately 500 uninfected $CD4^+$ cells can be killed by a single infected cell (Haseltine, 1988). Syncytium formation is facilitated by the interaction between glycoprotein gp120 on the virus-infected cell-membrane and its receptor protein CD4 residing on the membrane of the uninfected cells (Lifson et al., 1986; Sodroski et al., 1986). Inhibition of syncytium formation might, therefore, be limited to compounds interfering with the gp120-CD4 interaction such as dextran sulfate and the related polyanions, though it is more stringent than inhibition of HIV-1 cytopathogenicity (Baba et al., 1990).

Although AZT seems to improve the clinical and immunological status of patients with AIDS and AIDS-related complex (Fischl et al., 1987, 1990; Volberding et al., 1990; Yarchoan et al., 1986), AZT and 2′,3′-dideoxynucleoside analogs in general do not prevent syncytium formation (Mitsuya et al., 1988). The inhibitory effect of PM-19 on syncytium formation was reported previously (Take et al., 1991) and a similar behaviour is shown here for PM-104 (Table 2). These results support the hypothesis that polyoxometalates interfere with the virus adsorption (and/or penetration) process. Recently, dextran sulfate was reported to interact with positive charged amino acids in the V3 loop region of gp120 (Callahan et al., 1991). Due to the polyanionic nature of polyoxometalates, their anti-HIV-1 activity may result from reversible interaction with positive charged portions of either gp120, CD4 or both.

Like HPA-23, some polyoxometalates were found to inhibit the HIV-1 reverse transcriptase. However, there was no clear correlation between the anti-HIV-1 activity and enzyme inhibitory activity (Inouye et al., 1992).

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